
Sequence analysis

SChloro: directing *Viridiplantae* proteins to six chloroplastic sub-compartments

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Abstract

Motivation: Chloroplasts are organelles found in plants and involved in several important cell functions. Similarly to other compartments in the cell, chloroplasts have an internal structure comprising several sub-compartments, where different proteins are targeted to perform their functions. Given the relation between protein function and localization, the availability of effective computational tools to predict protein sub-organelle localizations is crucial for large-scale functional studies.

Results: In this paper we present SChloro, a novel machine-learning approach to predict protein sub-chloroplastic localization, based on targeting signal detection and membrane protein information. The proposed approach performs multi-label predictions discriminating six chloroplastic sub-compartments that include inner membrane, outer membrane, stroma, thylakoid lumen, plastoglobule and thylakoid membrane. In comparative benchmarks, the proposed method outperforms current state-of-the-art methods in both single- and multi-compartment predictions, with an overall multi-label accuracy of 74%. The results demonstrate the relevance of approach that is eligible as a good candidate for integration into more general large-scale annotation pipelines of protein subcellular localization.

Availability: The method is available as web server at <http://schloro.biocomp.unibo.it>

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1 Introduction

The eukaryotic cell hosts different compartments that play differentiated functional roles into the cell life cycle. Chloroplasts are organelles found in viridiplantae cells involved in crucial functions such as photosynthesis, fatty acid synthesis and immune response in plant organisms. Similarly to other compartments in the cell, such as the nucleus or mitochondria, in-depth experimental studies have identified several chloroplastic sub-compartments such as the envelope, the stroma, the thylakoid lumen and the membrane, in which proteins are targeted to perform different functions (Cooper and Hausman, 2009).

Few proteins found in the chloroplast are encoded by the organelle genome whereas the vast majority of them are nuclear encoded, synthesized by cytoplasmic ribosomes and then post-translationally targeted into the chloroplast by means of different mechanisms (Schleiff and Becker, 2010). Generally, targeting signals are present in the precursor protein and are used by the transport machinery to correctly direct the protein to its final destination. Most proteins directed to the stroma or to the enve-

lope carry a single cleavable N-terminal signal, while proteins directed to the thylakoid lumen and membrane are endowed with a bipartite signal, which provides information for the subsequent sorting of the protein from the stroma to the thylakoid. Furthermore, several non-cleavable sequence signals may also be present at any position along the sequence (typically membrane proteins are endowed with this type of signals) (Schleiff and Becker, 2010). In general, the import and sorting machinery is able to recognize these signals and transport both soluble proteins (directed to the stroma or the thylakoid lumen) and membrane proteins (directed to the thylakoid membrane or to the envelope) with single or multiple trans-membrane domains to their final working compartment (Schleiff and Becker, 2010).

Several computational tools have been developed so far to predict protein subcellular localization, given the impact of the feature on protein function characterization (Imai and Nakai, 2010).

The vast majority of available computational methods routinely discriminate macro compartments such as nucleus, cytoplasm, organelles and membranes. However, the prediction of more de-

tailed sub-localizations, such as the different sub-chloroplastic compartments, is challenging considering the paucity of detailed experimental annotations in publicly available databases (e.g., UniprotKB). For instance, only half of the currently available chloroplastic proteins with experimental evidence have also a sub-chloroplastic experimental annotation. Nonetheless, there has been a renewed interest in developing computational tools that are able to correctly identify very specific cellular sub-compartments (Kumar et al., 2014; Lin et al., 2013; Wang et al., 2015).

The prediction of sub-chloroplastic localization has been addressed in mainly two ways: (i) single-label approaches, which associates to the query protein a single localization compartment (Du et al., 2009; Tung et al., 2010; Shi et al., 2011; Hu and Yan, 2012) and (ii) multi-label approaches that can predict multiple localizations (Wang et al., 2015).

Generally, single-label methods consider four main chloroplastic sub-compartments: *envelope*, *stroma*, *thylakoid lumen* and *thylakoid membrane*. All of them are based on similar features extracted from protein sequence, which are then processed by different algorithms to perform the final prediction. SubChlo (Du et al., 2009), one the first released methods, is based on a variant of the k-nearest neighbor classifier and Chou's pseudo amino-acid composition (PseAAC) (Chou, 2001). In ChloroRF (Tung et al., 2010), a random forest classifier is fed with a protein encoding based on physicochemical properties extracted from the AAindex (Kawashima et al., 2008). SubIdent (Shi et al., 2011), which can also predict sub-mitochondrial localizations, performs predictions using SVMs and an alternative formulation of the PseAAC based on discrete wavelet transform. Finally, BS-KNN (Hu and Yan, 2012) is based on bit-score k-nearest neighbor and standard amino acid composition.

The only available multi-label method is MultiP-SChlo (Wang et al., 2015). It extends the set of possible compartments in which a protein can be found, by including the *plastoglobule*, lipoprotein particles present in all plastids. Then, using an algorithm based on multi-stage SVMs and PseAAC, the method performs multi-label predictions. MultiP-SChlo scores with an overall accuracy of 56% on a benchmark of multi-label dataset introduced in the same study (Wang et al., 2015).

In this paper we present SChloro, a novel machine-learning method to improve the prediction of protein sub-chloroplastic localization. The basic idea of our approach is to exploit the recognition of high-level topological and sorting features to improve the accuracy of the prediction of sub-chloroplastic localization. We adopt a two-stage prediction algorithm: first, we identify into the query protein, chloroplastic and/or thylakoid sorting signals, and second, we determine possible membrane interactions (suggesting membrane-related localizations). In the final step, these predicted features are integrated with global protein features to predict the final sub-chloroplastic localization, in a multi-label fashion. Differently from any previous approach, our method is able to provide predictions into six distinct compartments: inner membrane, outer membrane, stroma, plastoglobule, thylakoid lumen and thylakoid membrane. When compared to other state-of-the-art approaches, SChloro is able to significantly improve the prediction performance, scoring with a 74% overall multi-label accuracy. The method is available as web server at <http://schloro.biocomp.unibo.it>.

2 Methods

2.1 Datasets

In this study, three different datasets were used to evaluate the performance of our method and to compare it with previously developed approaches.

2.1.1 The SCEXP2016 dataset

The first dataset, referred to as SCEXP2016, was specifically compiled for this study and collects updated experimental data extracted from UniprotKB/SwissProt release 2016_01 (The Uniprot Consortium, 2014). In order to retain only high-quality data, the following procedure was adopted. Firstly, all chloroplastic proteins with experimentally annotated sub-cellular localization were extracted from UniprotKB/SwissProt. Only proteins with evidence at the protein level and longer than 50 residues were selected. From this initial set, to obtain very clean data, we filtered-out proteins that were annotated with additional localizations outside the chloroplast and retained only those with experimental annotation in at least one of the following six chloroplastic sub-compartments: inner membrane, outer membrane, stroma, plastoglobule, thylakoid lumen and thylakoid membrane. With this procedure, we ended up with 367 protein sequences, 309 of which are nuclear encoded whereas 26 are encoded by the chloroplastic genome (we decided to retain these proteins given the small number). Twenty-three out of 367 proteins are annotated with multiple chloroplastic sub-compartments (22 found in two compartments and 1 in three compartments).

Table 1. Distribution of proteins in SCEXP2016 into the six different chloroplastic sub-compartments

Compartment	Number of proteins
Inner membrane	47
Outer membrane	24
Stroma	119
Plastoglobule	32
Thylakoid lumen	37
Thylakoid membrane	131

Table 2. Distribution of annotated targeting and membrane features of SCEXP2016 proteins

Feature	Number of annotated proteins
Chloroplastic targeting	317
Thylakoid targeting	60
Single-pass membrane	34
Multi-pass membrane	62
Peripheral membrane	41

The distribution of proteins into the six different chloroplastic sub-compartments is summarized in Table 1. Furthermore, in Table 2 we also list the statistics of targeting signal and membrane interaction annotations (which will be used to train/test specific classifiers, as described in Section 2.4). It is worth to point out that, as detailed above, experimental evidence has been checked only for the primary annotation of proteins into subcellular compartments. In contrast, secondary protein annotation concerning targeting signals and membrane interaction were all retained and used as they were annotated for the selected proteins. As a consequence, these secondary annotations could be partially incomplete

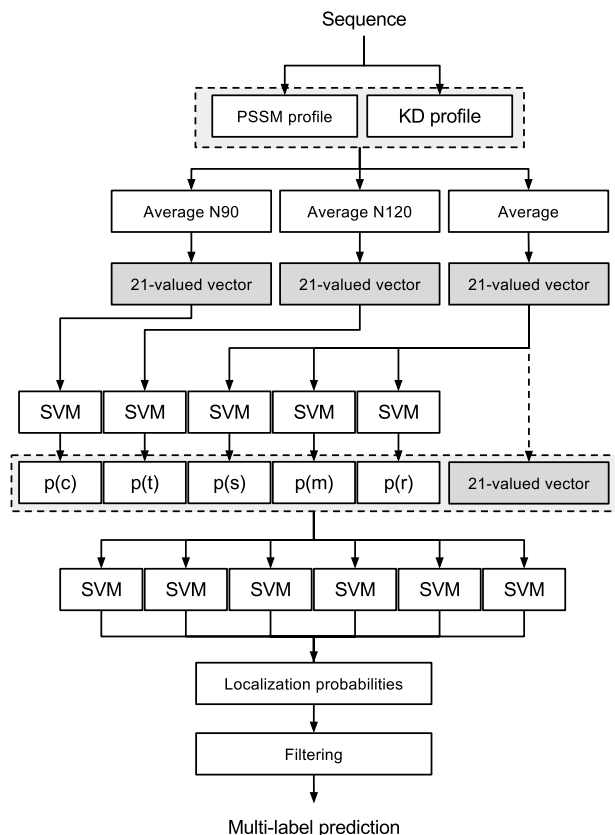


Figure 1. Overview of the SSchlo system architecture.

Homology clusters at 25% identity were identified using the blastclust program. These clusters were used to compile 10 cross-validation sets for the method evaluation. In particular, we avoided training/test bias by assigning all proteins in a cluster to the same cross-validation set.

2.1.2 The MSchlo578 dataset

The second dataset adopted in this study is the MSchlo578 dataset, previously released by Wang et al. (2015). This dataset contains 578 multi-compartment proteins distributed into the five following sub-chloroplastic localizations (in parenthesis the number of proteins): envelope (199), stroma (105), thylakoid lumen (34), thylakoid membrane (233) and plastoglobule (30). Twenty-two proteins are annotated with multiple sub-compartments (21 into two different compartments and 1 in three compartments). We used the MSchlo578 dataset to compare our method with the state-of-the-art method MultiP-Schlo (Wang et al., 2015).

2.1.2 The S60 dataset

Finally, a third dataset, referred to as S60 and introduced by Du et al. (2009), was used to compare our method with other methods in the single-label setting. The 262 proteins in this dataset are distributed among 4 different classes: envelope (40), stroma (71), lumen (44) and thylakoid membrane (129). No multiple annotations are reported for these proteins.

2.2 Sorting signals to chloroplast and its sub-compartments

Nuclear encoded chloroplastic proteins are targeted toward the organelle by means of biological pathways involving the molecular recognition of

specific sorting signals (Schleiff and Becker, 2010). At the higher level, precursor proteins synthesized by cytoplasmic ribosomes are endowed with the well-known transit peptide, a variable-length stretch of sequence located at the N-terminus of the nascent protein (Schleiff and Becker, 2010; Bruce, 2001; Patron and Waller, 2007). Once the protein reaches its destination into the chloroplast (typically the stroma), the transit peptide is cleaved by specific proteins. Some chloroplastic proteins of the thylakoid lumen and membranes are endowed with an additional signal located immediately after the transit peptide. This thylakoid transit peptide is used for the subsequent protein sorting from the stroma to the thylakoid (Schleiff and Becker, 2010; Bruce, 2001).

In addition, a subset of nuclear-encoded chloroplastic proteins was found as not having the classic transit peptide. These proteins are mainly outer-membrane proteins (and also inner-membrane and inter-membrane space proteins, although to a lesser extent) with alpha helical membrane anchors, which also carry targeting information (Schleiff and Klösgen, 2001; Soll, 2002).

In this paper we try to exploit the knowledge about these mechanisms by defining signal-specific detectors and integrating them into our localization prediction system (see Section 2.4 for details).

2.3 Membrane interaction

The structure of the chloroplasts comprises three different membrane systems: the inner and outer membranes and the thylakoid membrane system. The inner and outer membranes form the chloroplast *envelope*, which borders the *stroma*, and separates it from the cytoplasm. Inside the stroma, it is found the thylakoid, an additional membrane-bounded structure. The *thylakoid membrane* separates the stroma from the *lumen*. Several membrane proteins with diverse topologies can be found as either directly or indirectly interacting with the three membrane systems. According to the type of interaction, three major classes can be distinguished:

- (1) *Integral single-pass membrane* proteins, which spans the membrane with a single trans-membrane domain.
- (2) *Integral multi-pass membrane* proteins, which spans the membrane with multiple trans-membrane domains.
- (3) *Peripheral membrane* proteins, which do not span the membrane and interact with it through different mechanisms including lipid anchoring, direct interaction with the phospholipid bilayer through specific domains or indirect interaction through integral membrane proteins.

From the point of view of protein sub-chloroplastic localization prediction, knowing if a protein interact with a membrane or not may directly restrict the number of possible compartments it may be found in. Furthermore, the precise knowledge of the interaction type (single, multi pass or peripheral) may give some additional insight about the final destination of the protein. In this paper, we exploited these considerations by integrating membrane-interaction specific classifiers into our localization prediction system (see Section 2.4 for details).

2.4 Overview of the prediction method

The proposed multi-label prediction system, depicted in Figure 1, consists of two layers of Support Vector Machines (SVMs). Classifiers of the first layer are devised to predict the occurrence probabilities of *chloroplast* and/or *thylakoid* sorting signals as well as the probabilities for the protein to be in one of three possible interaction states with a membrane (*single*-, *multi-pass* trans-membrane or *peripheral* membrane protein). Therefore, five different classifiers were defined: two for the sorting signals and three for the membrane interaction. Each classifier was trained using available experimental evidence and slightly different input

features optimized for the specific feature prediction task. In particular the following input features are used here:

- 1) The average composition of the Position Specific Scoring Matrix (PSSM) as computed from the multiple sequence alignment obtained using the psi-blast program (Altschul et al., 1997) to search the query sequence against the UniProtKB/SwissProt database (The UniProt Consortium, 2014). Raw PSSM values are rescaled before averaging into the range [0,1] using a standard logistic function $1/(1+e^{-(x)})$. In this way, the average PSSM consists of a 20-valued vector with elements ranging between 0 and 1.
- 2) The average hydrophobicity computed along the protein sequence using the Kyte-Doolittle scale (Kyte and Doolittle, 1982). Hydrophobicity values are firstly linearly rescaled before averaging into the range [0,1] so that the highest and the lowest values, namely 4.5 and -4.5 for isoleucine and arginine, map to 0 and 1, respectively. Hence, the average hydrophobicity feature consists of a single real value between 0 and 1.

For the chloroplast and thylakoid targeting classifiers, considering that the two targeting signals are located at the N-terminus of the protein, the first 90 and 120 residues were used to compute the average values, respectively. In contrast, the entire protein sequence has been used for the three membrane interaction classifiers. Altogether, the first layer outputs are collected into a 5-valued vector defined as follows:

$$[p(c), p(t), p(s), p(m), p(r)] \quad (1)$$

where the first two values are, respectively, the probabilities of having a chloroplastic-targeting signal ($p(c)$) and thylakoid-targeting signal ($p(t)$), while the last three values are the probabilities for the protein to be, respectively, a single-pass ($p(s)$), a multi-pass ($p(m)$) and a peripheral ($p(r)$) membrane protein.

The second layer of SVM classifiers computes the membership probability for the query protein to be located into one or more sub-chloroplastic compartments.

One separate classifier was defined for each localization compartment. Each second-layer classifier was trained using a 26-valued feature vector consisting of: (i) the 5-valued vector as defined in Eq. 1, and (ii) the average PSSM and hydrophobicity both computed on the entire protein sequence. The final, multi-label prediction is obtained by taking all SVM outputs whose probability output is greater or equal to 0.5.

Adopting this two-layered architecture allows a better exploitation of different basic features that are computed over different portions of the sequence. By this, an intermediate representation of the protein in terms of presence/absence of sorting signals as well as interaction with the membrane, is computed.

2.2 Model selection and implementation

The method evaluations are carried-out using either a 10-fold cross-validation procedure (to train/test our method on the SCEXP2016 dataset), or by adopting a jackknife test (to compare with other methods in literature on the MSchlo78 and S60 datasets). Regardless of the performed actual evaluation setting, the benchmark procedure needs to be carefully tuned to deal with the specific structure of our prediction system that comprises two cascading levels of classifiers.

To achieve this, we applied the following procedure. First of all, for each cross-validation or jackknife run, a fraction of the training set was extracted and used as a validation set. This set was used to adjust hyperparameters as well as to identify the optimal input feature encoding for both first- and second-layer classifiers. Once selected, these hyperparameters were frozen and used to predict the remaining testing data.

SVM classifiers were implemented using the standard libsvm software package (Chang et al., 2011). Each classifier is based on a non-linear Radial Basis Function (RBF) kernel and is trained/tested to provide

probabilistic outputs using the standard model implemented by the software library.

Concerning the cascading structure, optimal first-layer classifiers (found through validation sets) were used to generate both training/testing data for second-layer classifiers. In this way, SVMs of the second-layer were trained/tested on predicted values and this allowed evaluating the entire pipeline taking into account the potential error propagation between the two layers.

2.3 Scoring measures

For sake of comparison with different methods available in literature, our system was evaluated using either multi-label or single-label scoring measures. More formally, let y_i and p_i be the set of observed and predicted labels (compartments) for the i^{th} protein, and let n be the total number of proteins in the dataset. To score the prediction performance in the multi-label setting, we adopted the following scoring indexes (Wang et al., 2015):

- The multi-label Accuracy (mlACC), defined as:

$$mlACC = \frac{1}{n} \sum_{i=1}^n \frac{|y_i \cap p_i|}{|y_i \cup p_i|} \quad (2)$$

- The multi-label Recall (mlREC), defined as:

$$mlREC = \frac{1}{n} \sum_{i=1}^n \frac{|y_i \cap p_i|}{|y_i|} \quad (3)$$

- The multi-label Precision (mlPRE), defined as:

$$mlPRE = \frac{1}{n} \sum_{i=1}^n \frac{|y_i \cap p_i|}{|p_i|} \quad (4)$$

- The multi-label F1 (mlF1), defined as:

$$mlF1 = \frac{2 \cdot mlREC \cdot mlPRE}{mlREC + mlPRE} \quad (5)$$

- The overall multi-label accuracy (ACC^{ml}), defined as:

$$ACC^{ml} = \frac{1}{n} \sum_{i=1}^n \mathbf{1}(y_i \equiv p_i) \quad (6)$$

where $\mathbf{1}(y_i \equiv p_i)$ is an indicator function that equals to 1 if the two sets are identical, 0 otherwise.

To score the prediction performance in the single-label setting we used the following scoring indexes (Du et al., 2009):

- The single-label accuracy of label l ($ACC^{sl}(l)$), defined as:

$$ACC^{sl}(l) = \frac{TP_l}{TP_l + FN_l} \quad (7)$$

- The overall single-label accuracy (ACC^{sl}), defined as:

$$ACC^{sl} = \frac{1}{n} \sum_{l=1}^m TP_l \quad (8)$$

where TP_l and FN_l are true positive and false negatives for the label l , respectively, and m is the number of different labels.

3 Results

3.1 Single- and multi-label performance of SChloro on the SCEXP2016 dataset

Table 3 lists the 10-fold cross-validation results obtained using different input features and evaluated on the SCEXP2016 dataset. Both single- and multi-label scoring indexes are reported. The baseline predictor (first row in Table 3) does not include information about targeting signals and membrane interaction

Table 3. Single- and multi-label performance with different combinations of input features on the SCEXP2016 dataset by adopting a 10-fold cross-validation procedure.

Input features	Multi-label prediction					Single-label prediction						
	ACC ^{ml}	mIACC	mIPRE	mIREC	mIF1	ACC ^{sl} (I)	ACC ^{sl} (O)	ACC ^{sl} (S)	ACC ^{sl} (L)	ACC ^{sl} (M)	ACC ^{sl} (P)	ACC ^{sl}
Basic	0.50	0.57	0.59	0.63	0.61	0.33	0.26	0.65	0.65	0.60	0.52	0.60
Basic+target+mem (predicted)	0.65	0.79	0.80	0.94	0.86	0.66	0.63	0.91	0.92	0.84	0.81	0.92
Basic+target+mem (observed)	0.79	0.87	0.89	0.94	0.91	0.79	0.75	0.99	1.0	0.92	0.89	0.97

Basic=PSSM+Hydrophobicity; target=[p(c),p(t)]; mem=[p(s),p(m),p(r)]. Scoring indexes are defined as in Section 2.3. In single-label scoring indexes, I, O, S, L, M and P stand for inner membrane, outer membrane, stroma, thylakoid lumen, thylakoid membrane and plastoglobule, respectively.

and it was trained/tested using the basic feature encoding (consisting of average PSSM and hydrophobicity computed on the entire protein sequence. In this case, only the second layer of the SVM system is used).

When predicted probabilities of targeting signals and membrane interaction are included, the prediction performance significantly improves (compare rows 1 and 2, in Table 3). In particular, we observe a general improvement in performance, with ACC^{ml} increasing up to 0.65 and ACC^{sl} up to 0.92. Furthermore, also individual single-label accuracies improve, suggesting a general positive contribution of the five predicted features.

For sake of comparison, we also report results obtained when the real information about targeting signals and membrane interaction is included in the second step of the procedure (i.e. in both training and testing, predicted probabilities are replaced by binary features derived from the true annotation of each protein). The reported performance scores represent the maximum theoretical accuracy that can be achieved on this dataset assuming a perfect targeting and membrane interaction prediction. This theoretical predictor achieves very high overall accuracies (ACC^{ml}=0.79 and ACC^{sl}=0.97), suggesting that the proposed approach builds on top of sound bases and that the prediction performance might be further improved by providing more accurate first-level feature predictors.

Table 4. 10Fold cross-validation performance of SChloro classifiers for targeting signals and membrane interactions

Classifier	AUC	MCC
Chloroplast targeting	0.96	0.85
Thylakoid targeting	0.95	0.76
Single-pass membrane	0.88	0.51
Multi-pass membrane	0.95	0.79
Peripheral membrane	0.82	0.44

Finally, for sake of completeness, in Table 4 we also report the performance of individual first-layer classifiers devised to predict sorting signals and membrane interaction. Considering the results and the inherent difficulty of each prediction task, it ap-

pears that the effectiveness of individual predictors is strongly affected by the corresponding abundance of the annotated data in the dataset (compare Tables 2 and 4).

3.2 Comparison with other single- and multi-label methods

In Table 5 we report a comparative benchmark of different methods on the S60 dataset (Du et al., 2009). For sake of comparison, results of SChloro were computed using a jackknife test, while performance scores for other methods were taken from literature (Wang et al., 2015). In particular, we report overall single-label accuracies using the same annotation scheme consisting of four different labels (E=envelope, S=stroma, L=lumen, M=thylakoid membrane), respectively, for our method and for other five different single-label methods available in literature: SubChlo (Du et al., 2009), ChloroRF (Tung et al., 2010), SubIdent (Shi et al., 2011), BS-KNN (Hu and Yan, 2012) and MultiP-Schlo (Wang et al., 2015). The results indicate that SChloro provides in general more balanced predictions compared to others. Other methods tend to over-predict the more abundant labels in the dataset (i.e. thylakoid membrane and stroma), whereas SChloro scores, on average, better on overall accuracy and in all the remaining compartments (e.g. compare accuracy results for the lumen and envelope labels).

Table 5. Comparison of single-label performance of different methods on the S60 dataset adopting a jackknife test.

Method	ACC ^{sl}	ACC ^{sl} (E)	ACC ^{sl} (S)	ACC ^{sl} (L)	ACC ^{sl} (M)
SChloro	0.90	0.93	0.96	0.98	0.89
MultiP-Schlo*	0.89	0.73	0.96	0.61	1.0
SubChlo*	0.67	0.40	0.67	0.43	0.84
ChloroRF*	0.67	0.48	0.57	0.39	0.88
SubIdent*	0.89	0.80	0.86	0.64	0.98
BS-KNN*	0.76	0.48	0.74	0.78	0.85

Scoring indexes are defined in Section 2.3. Labels E, S, L, and M stand for envelope, stroma, thylakoid lumen and thylakoid membrane, respectively. * Data taken from Wang et al., (2015)

Finally, multi-label prediction performances are reported in Table 6. Here we compare SChloro with MultiP-Schlo (Wang et al., 2015). In this case, results reported for our method are computed using the same annotation scheme of MultiP-Schlo, including five compartments: envelope, stroma, lumen, thylakoid membrane and plastoglobule. In this benchmark, we obtain a significant improvement. SChloro outperforms MultiP-Schlo in all scoring indexes reported, achieving an improvement of about 12% in overall multi-label accuracy.

Table 6. Comparison of multi-label performance of MultiP-Schlo and our method on the MSchlo578 dataset

Method	ACC ^{ml}	mlACC	mlPRE	mlREC	mlF1
SChloro	0.74	0.76	0.78	0.78	0.78
MultiP-Schlo	0.56	0.63	0.64	0.71	0.67

Scoring indexes are defined in Section 2.3. The comparison adopts a jackknife test.

Conclusion

Assessing the protein sub-cellular localization is an important step toward protein function prediction. The rapid pace at which new proteomes become available through NGS technologies requires the availability of effective computational tools for assessing protein localization and function to fill the gaps of the experimental knowledge.

In this paper we presented SChloro, a novel approach to predict protein sub-chloroplastic localization into six main compartments including inner and outer membranes, stroma, plastoglobule, lumen and thylakoid membrane. Our method is based on the recognition of sequence signals that define target specificity (chloroplast and thylakoid targeting signals) as well as on the prediction of the potential type of interaction with chloroplast membranes (single-pass, multi-pass and peripheral interaction). We show that this information can be profitably incorporated into a two-level SVM-based algorithm to predict both single and multiple protein sub-chloroplastic localizations with high accuracy. In fact, SChloro significantly outperforms the available state-of-the-art methods, both in single and multi-label settings. Furthermore, our method introduces the possibility of discriminating six different types of sub-chloroplast localization. This suggests SChloro as a good candidate for the integration into a more comprehensive pipeline for the annotation of sub-cellular localization of protein in viridiplant organisms.

The complete prediction system is available as web-server at <http://schloro.biocomp.unibo.it>.

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